CLAIMS

1. A composition consisting essentially of RlmA protein in crystalline form, wherein the RlmA is $RlmA^{I}$ or $RlmA^{II}$.

2. A method for screening compound libraries to identify compounds which bind in the cleft of an RlmA protein in crystalline form, wherein said RlmA is $RlmA^{I}$ or $RlmA^{II}$, and which compounds are potential inhibitors of rRNA-binding or methylation function, comprising X-ray crystallography.

3. The method of claim 2, wherein the compound is soaked into crystal of RlmA or co-crystallized with RlmA molecules in order to identify a compound that binds at the RNA binding site of RlmA.

4. The method of claim 2, comprising detecting interference with the function of RlmA by inhibiting S-adenosylmethionine-binding or other aspects of the catalytic mechanism of the methyltransferase domain.

5. The method of claim 2, comprising identification of the orientations and binding modes of said compounds, for structure-based drug design.

6. A method comprising the use of three-dimensional coordinates of a model for the RlmA:rRNA complex for designing compound libraries for screening.

7. A method of identifying a compound that can be used to treat bacterial infections, either alone or in combination with other antibiotics, comprising identifying a compound for use as

an inhibitor of the RlmA, or an rRNA binding domain thereof, and a dataset comprising the three-dimensional coordinates obtained from the RlmA, or an rRNA binding domain thereof.

8. The method of claim 7, wherein the identification of a compound is performed in conjunction with computer modeling.

9. The method of claim 7, further comprising the three-dimensional coordinates of the RlmA and the model of the RlmA:rRNA complex provide methods for (a) designing an inhibitor library for screening, (b) rational optimization of lead compounds, and (c) virtual screening of potential inhibitors.

10. A composition comprising a reaction mixture comprising a complex of a bacterial RlmA protein, or an rRNA binding fragment thereof, and an rRNA fragment that binds said protein.

11. The composition of claim 10, comprising an rRNA binding domain of said RlmA protein.

12. The composition of claim 10, wherein the rRNA binding domain is rRNAhp35.

13. The composition of claim 10, further comprising a compound being tested for inhibitory activity against a bacterial strain.

14. The composition of claim 10, wherein the bacterial RlmA protein or the rRNA is detectably labeled.

15. A method of identifying compounds having inhibitory activity against a bacterial strain, or an ability to interfere with the interaction between RlmA and the rRNA *in vitro*, comprising:

a) preparing a reaction system comprising a bacterial RlmA protein or a rRNA binding domain thereof, a rRNA that binds said protein or binding domain thereof, and a candidate compound; and

b) detecting extent of binding between the bacterial RlmA protein and the rRNA, wherein reduced binding between the bacterial RlmA protein and the rRNA in the presence of the compound relative to a control is indicative of inhibitory activity of the compound against the bacterial strain or the ability of the compound to interfere with the interaction between RlmA and the rRNA.

16. The method of claim 15, wherein the compounds have inhibitory activity against RNA-binding and S-adenosylmethionine binding in a bacterial strain, and wherein further the compounds can be linked by a flexible linker group to design more effective inhibitors.

17. The method of claim 15, wherein the bacterial RlmA protein or rRNA binding domain thereof is immobilized on a solid support.

18. The method of claim 15, wherein the candidate compound is added to the reaction system prior to or simultaneously with the bacterial RlmA protein and the rRNA.

19. The method of claim 15, wherein the candidate compound is added to the reaction system subsequent to addition of the bacterial RlmA protein and the rRNA.

20. The method of claim 15, further comprising labeling the

rRNA, rRNA fragment, bacterial RlmA protein or rRNA binding domain thereof with a detectable label, prior to said detecting.

21. The method of claim 15, wherein the method of identifying compounds having inhibitory activity is selected from the group consisting of (a) NMR chemical shift perturbation of the RlmA protein, (b) gel filtration chromatography, c) sedimentation equilibrium measurements using an analytical ultracentrifuge, (d) sedimentation velocity measurements, (e) amide hydrogen-deuterium exchange measurements using NMR or mass spectrometry, (e) static light scattering measurements,(f) dynamic light scattering measurements, or (g) virtual screening using the structure of the RlmA protein and the model of the RlmA protein-rRNA complex.

22. The method of claim 15, wherein the method of identifying compounds having inhibitory activity comprises NMR chemical shift perturbation measurements conducted in the absence of resonance assignments to detect RlmA-rRNA interactions, wherein the compounds are identified which prevent these interactions.

23. The method of claim 20, wherein the detectable label comprises an antibody or fragment thereof that binds the bacterial RlmA protein or rRNA binding domain thereof.

24. The method of claim 20, wherein the detectable label comprises an enzyme and the reaction system further comprises a substrate for the enzyme.

25. The method of claim 20, wherein the detectable label

comprises a radioisotope.

26. The method of claim 20, wherein the detectable label comprises a fluorescent label.

27. The method of claim 20, wherein said detecting is conducted via fluorescent resonance energy transfer.

28. The method of claim 20, wherein said detecting is conducted via fluorescence polarization anisotropy measurements.

29. The method of claim 15, wherein the bacterial RlmA protein or rRNA binding fragment thereof is present in a reaction system selected from the group consisting of (a) a fusion protein with glutathione-S-transferase assay, (b) a fluorescence-detected hpt screening assay, (c) a virtual screening assay using a three-dimensional structure of E coli $RlmA^{I}$, models of $RlmA^{I}$ or $RlmA^{II}$ proteins and/or models of RlmA-rRNA complexes based on the coordinates of E coli RlmAI, and (d) a high throughput screening assay using a library that is biased based on the three-dimensional structure of E coli $RlmA^{I}$, models of $RlmA^{I}$ or $RlmA^{II}$ proteins and/or models of RlmA-rRNA complexes based on the coordinates of E coli RlmAI.

30. The method of claim 15, wherein the method of identification comprises a high throughput screening assay.

31. A method of preparing a composition for inhibiting replication of a bacterial strain *in vitro* or *in vivo*, comprising:

a) preparing a reaction system comprising a bacterial RlmA protein or a rRNA binding domain thereof, a rRNA that

binds said protein or binding domain thereof, and a candidate compound;

b) detecting extent of binding between the bacterial RlmA protein and the rRNA, wherein reduced binding between the bacterial RlmA protein and the rRNA in the presence of the compound relative to a control is indicative of inhibitory activity of the compound against the bacterial strain; and

c) determining extent of a compound identified in b) as having activity to inhibit growth of a bacterial strain *in vitro*, either alone or in the presence of another antibiotic.

32. The method of claim 31, wherein the RlmA protein is an RlmA protein or an rRNA binding domain thereof.

33. The method of claim 31, wherein the rRNA is rRNAhp35 or other RNA fragments.

34. The method of claim 31, wherein the co-antibiotic is a macrolide antibiotic.

35. A method for identifying a compound that binds to a bacterial RlmA protein in a first entity, comprising the steps of: (a) preparing a reaction solution including the compound to be tested and a first entity including a bacterial RlmA protein; and (b) detecting at least one of the presence, extent, concentration-dependence, or kinetics of binding of the compound to the bacterial RlmA protein.

36. The method of claim 35 wherein the first entity is an

intact bacterial RlmA.

37. The method of claim 35 wherein the first entity is a fragment of a bacterial RlmA.

38. The method of claim 35 wherein the first entity is *Escherichia coli* RlmA or a derivative thereof.

39. The method of claim 35 further comprising the step of: detecting at least one of the presence, extent, concentration-dependence, or kinetics of binding of the compound to a second entity that contains a derivative of a bacterial RlmA protein having at least one amino acid substitution, insertion, or deletion.

40. The method of claim 39 wherein the second entity is a derivative of an intact bacterial RlmA.

41. The method of claim 39 wherein the second entity is a derivative of a fragment of a bacterial RlmA.

42. The method of claim 39 wherein the second entity is a derivative of *Escherichia coli* RlmA.

43. A method of designing compounds which bind to the rRNA-binding pocket of RlmA, comprising modifying rRNAhp35 to synthesize analogs thereof.

44. A method for identifying a compound that inhibits an activity of a bacterial RlmA by binding to a bacterial RlmA protein, comprising: (a) preparing a reaction solution comprising the compound to be tested and a first entity containing a bacterial RlmA protein; and (b) detecting at least one of the presence, extent, concentration-dependence, or kinetics of

inhibition of an activity of said first entity, wherein inhibition involves binding of the compound to the bacterial RlmA.

45. The method of claim 44, wherein inhibition involves binding of the compound to rRNA or rRNA fragments thereof.

46. The method of claim 44 wherein the first entity is an intact bacterial RlmA.

47. The method of claim 44 wherein the first entity is a fragment of a bacterial RlmA.

48. The method of claim 44 wherein first entity is *Escherichia coli* RlmA or a derivative thereof.

49. The method of claim 44 wherein the activity is RNA binding.

50. The method of claim 44 further comprising the step of: detecting at least one of the presence, extent, concentration-dependence, or kinetics of the inhibition by the compound of the activity of a second entity that contains a derivative of a bacterial RlmA protein sequence having at least one amino-acid substitution, insertion, or deletion.

51. The method of claim 50 wherein the second entity is a derivative of an intact bacterial RlmA A.

52. The method of claim 50 wherein the second entity is a derivative of a fragment of a bacterial RlmA.

53. The method of claim 50 wherein the second entity is *Escherichia coli* RlmA or a derivative thereof.

54. The method of claim 50 wherein the activity is RNA

binding.

55. The method of claim 50 wherein inhibition of an activity of the first entity and inhibition of an activity of the second entity are assessed sequentially.

56. The method of claim 50 wherein inhibition of an activity of the first entity and inhibition of an activity of the second entity are assessed simultaneously.

57. The method of claim 50 wherein at least one of the presence, extent, concentration-dependence, or kinetics of inhibition by the compound of an activity of the first entity also is compared to at least one of the presence, extent, concentration-dependence, or kinetics of inhibition by an inhibitory compound specific to the bacterial RlmA protein of an activity of the second entity.

58. A method for identifying a compound that binds to a bacterial RlmA protein, comprising (a) preparing a reaction solution comprising the compound to be tested, a first entity containing a bacterial RlmA protein, and containing a detectable group within RlmA protein; and (b) detecting a change in a property of the detectable group within RlmA protein.

59. The method of claim 58 wherein the first entity is an intact bacterial RlmA protein.

60. The method of claim 58 wherein the first entity is a fragment of an RlmA protein.

61. The method of claim 58 wherein the first entity is *Escherichia coli* RlmA or a derivative thereof.

62. The method of claim 58 wherein the compound to be tested contains a chromophore.

63. The method of claim 58 wherein the detectable group contains a fluorophore.

64. The method of claim 58 further comprising measurement of fluorescence resonance energy transfer.

65. The method of claim 58 wherein the detectable group is an NMR-active ^{1}H, ^{13}C or ^{15}N nucleus.

66. The method of claim 58 further comprising the step of: detecting at least one of the presence, extent, concentration-dependence, or kinetics of the binding of the compound to a second entity that contains a derivative of a bacterial RlmA protein having at least one amino-acid substitution, insertion, or deletion.

67. The method of claim 66 wherein the second entity is a derivative of an intact bacterial RlmA protein.

68. The method of claim 66 wherein the second entity is a derivative of a fragment of a bacterial RlmA protein.

69. The method of claim 66 wherein the second entity is *Escherichia coli* RlmA or a derivative thereof.

70. A method for identifying a compound that binds to a bacterial RlmA protein, comprising (a) preparing a reaction solution comprising the compound to be tested, a first entity containing a bacterial RlmA protein, a second entity containing an rRNA, or rRNA fragment or variant thereof, specific to the rRNA-binding pocket of the RlmA protein, and containing a

detectable group within the rRNA; and (b) detecting a change in a property of the detectable group within rRNA.

71. The method of claim 71, wherein the first entity is an intact bacterial RlmA protein.

72. The method of claim 71, wherein the first entity is a fragment of an RlmA protein.

73. The method of claim 71 wherein the first entity is *Escherichia coli* RlmA or a derivative thereof.

74. The method of claim 71 wherein the compound to be tested contains a chromophore.

75. The method of claim 71 wherein the detectable group contains a fluorophore.

76. The method of claim 71 wherein the detectable group is an NMR-active ^{1}H, ^{13}C or ^{15}N nucleus.

77. The method of claim 71 further comprising an assay selected from the group consisting of a measurement of fluorescence resonance energy transfer, changes in absorbance, steady-state fluorescence, time-resolved fluorescence, fluorescence polarization anisotropy, or NMR spectroscopy.

78. A method for identifying a compound that binds to a bacterial RlmA protein, comprising (a) preparing a reaction solution comprising the compound to be tested, a reference compound that binds to a bacterial RlmA protein and a first entity containing a bacterial RlmA protein, and (b) detecting at least one of the presence, extent, concentration-dependence, or kinetics of competition by the compound for binding of the

reference compound to the RlmA protein.

79. The method of claim 78 wherein the first entity is an intact bacterial RlmA protein.

80. The method of claim 78 wherein the first entity is a fragment of a bacterial RlmA protein.

81. The method of claim 78 wherein the first entity is *Escherichia coli* RlmA protein or a derivative thereof.

82. The method of claim wherein the reference compound is selected from the group consisting of a rRNA, a fragment thereof, a variant thereof or rRNAhp35.

83. The method of claim 78 wherein the reference compound contains a detectable group.

84. The method of claim 78 wherein the detectable group contains a chromophore.

85. The method of claim 78 wherein the detectable group contains a fluorophore.

86. The method of claim 78 wherein the reference compound is a chromophore-labeled inhibitory compound specific to the bacterial RlmA protein.

87. The method of claim 78 wherein the reference compound is a fluorophore-labeled inhibitory compound specific to the bacterial RlmA protein.

88. The method of claim 78 further comprising measurement of fluorescence resonance energy transfer.

89. The method of claim 78 wherein displacement of the reference compound is assessed by NMR spectroscopy.

89. The method of claim 78 further comprising the step of: detecting at least one of the presence, extent, concentration-dependence, or kinetics of the binding of the compound to a second entity that contains a derivative of a bacterial RlmA protein having at least one amino acid substitution, insertion, or deletion.

90. The method of claim 78 wherein the second entity is a derivative of an intact bacterial RlmA protein.

91. The method of claim 78 wherein the second entity is a derivative of a fragment of a bacterial RlmA protein.

92. The method of claim 78 wherein the second entity is *Escherichia coli* RlmA protein or a derivative thereof.

93. The method of claim 78 wherein at least one of the presence, extent, concentration-dependence, or kinetics of binding of the compound to the first entity is compared to at least one of the presence, extent, concentration-dependence, or kinetics of binding of an inhibitory compound specific to the bacterial RlmA protein to the second entity.